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Buxtonella Sulcata A Ciliate

Associated with Ulcerative Colitis in a Cow and Prevalence of Infection in Nebraska Cattle

H. D. Urman, D.V.M., Ph.D. and G. W. Kelley, M.S., Ph.D.

INTRODUCTION

The ciliated protozoan, *Buxtonella sulcata*, was found in 28% of a sample of Iowa cattle in 1927 (2), and in 32% of a sample of Louisiana cattle in 1930 (5). As far as we can ascertain it has not been reported in American cattle since. During the summer of 1962, *B. sulcata* was found associated with ulcerative colitis in a cow from northeast Nebraska. This finding prompted an investigation of the present incidence of infection in the region.

METHODS

Fecal examinations were done on eight herds of cattle. Samples were collected randomly from freshly passed feces being careful to avoid soil contamination. Usually twelve samples were collected from each herd regardless of the number of animals in the group.

The presence of *B. sulcata* cysts was determined by microscopic examinations of direct smears, sedimentation, or flotation concentration preparations. The direct smears were made by placing a small portion of feces on a clean slide, mixing it into a drop of water, adding a cover

slip, and examining with a compound microscope. The sedimentation method consisted of emulsifying one gram of feces in 10 ml water, straining to remove coarse particles; centrifuging (2,000 rpm, 2 min); decanting; adding water to 2 ml volume; resuspending the sediment; and counting cysts in three separate drops with aid of microscope. Quantitative counts were possible with this method. Flotation concentration was conducted using saturated sodium nitrate solution by modification of the Stoll-Lane direct centrifugal flotation method, Stoll (6). Zinc sulfate and sugar solutions did not give as good results as sodium nitrate. Most of the samples were examined by this flotation method.

The ulcerative colitis associated with *B. sulcata* was found in an adult dairy cow which was submitted to the University of Nebraska Veterinary Science Department for post mortem examination. The animal came from northeast Nebraska and had died of water hemlock poisoning. She had been sick for three weeks prior to death.

Along with other tissues, pieces of large intestine were fixed in buffered neutral 10% formalin. The fixed tissue was embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin. Periodic acid Schiff (PAS) technique was applied for histochemical determination of carbohydrates in the parasites.

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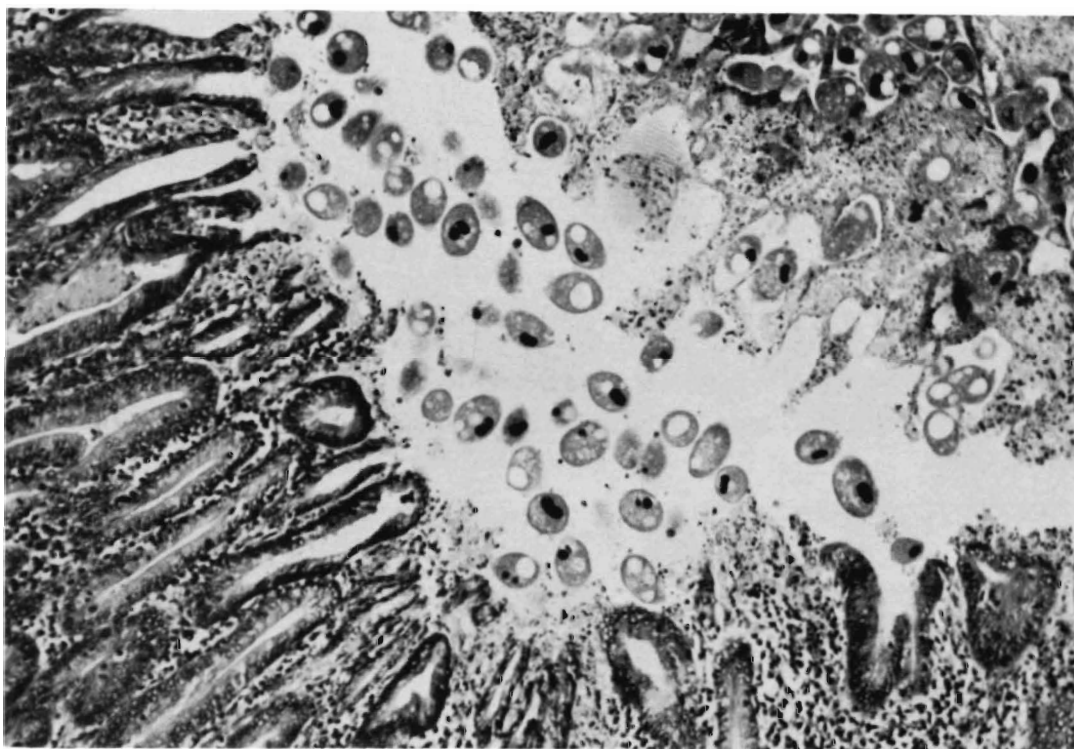


Figure 1. *B. sulcata* associated with necrotic material within the crater of an ulcer. Hematoxylin eosin stain. 80X

RESULTS

The mucosa of the colon was congested and contained many small ulcers. Each ulcer was covered with necrotic material and the craters had undermined edges.

Microscopically, the necrotic material stained strongly eosinophilic. Beneath the necrotic covering there was an inflammatory reaction that extended deep beneath the villi and occasionally into the submucosa. The cellular infiltration was made up of histiocytes and lymphocytes. Polymorphonuclear neutrophils and eosinophils were rare.

The parasites were usually located between the necrotic material and the inflamed tissue. They were occasionally found deep in the inflamed mucosa of the colon (Fig. 1). Clusters of ciliates were also observed in the lymphatics and serosa of the colon (Fig. 2); there was no tissue reaction in the serosa.

The parasites within the fixed and stained sections were oval to round depending on the plane of sectioning; they averaged $53\ \mu \times 31\ \mu$ in size. The body was enveloped with a thin pellicle covered with cilia—still visible in this formalin fixed material (Fig. 3). A cytostome was observed anteriorly. The cytoplasm stained slightly basophilic and contained numerous food vacuoles with phagocytized red blood cells and cellular debris. Similar phagocytized objects were observed within the parasites which were found in the serosa. One or two large empty vacuoles were observed in each parasite. A large kidney-shaped nucleus was located eccentrically. When stained with PAS technique, the cytoplasm was observed to be filled with densely packed purplish-red, minute granules (carbohydrates) (Fig. 4). Degenerating parasites did not stain with PAS.

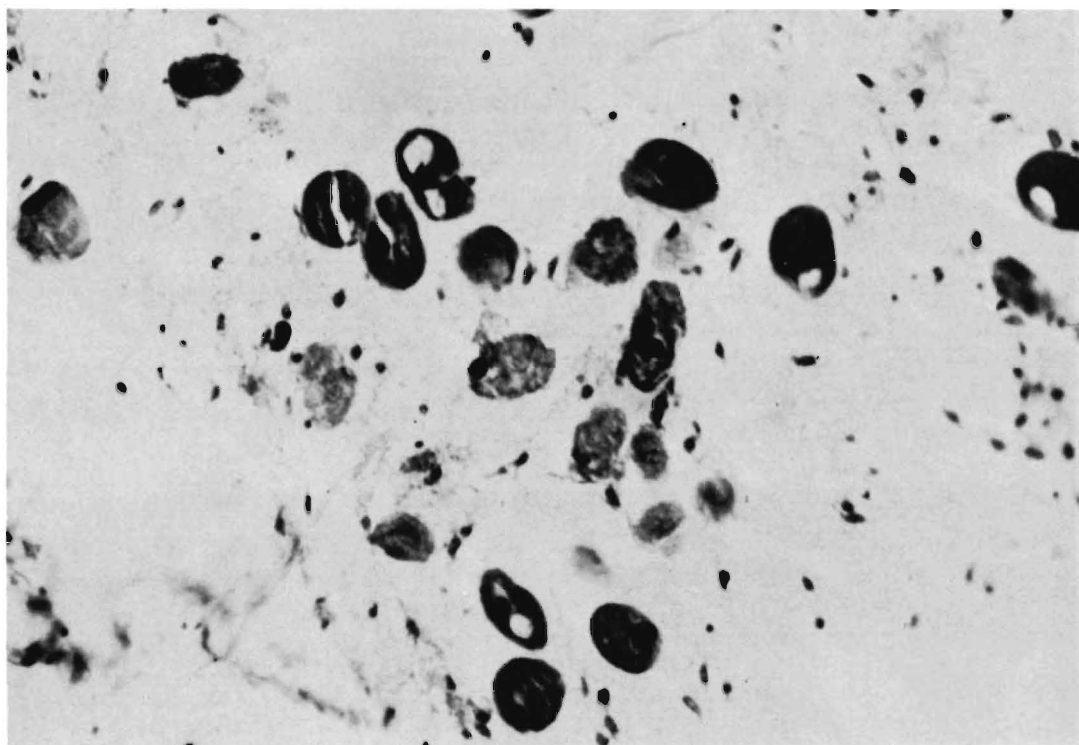


Figure 2. Parasites probably migrated into the serosa post mortem since there is no tissue reaction associated with their presence. 120X

Composition of the herds examined and incidence of *B. sulcata* is presented in Table 1. The incidence ranged from 58% infection in a herd of range yearlings to zero in two groups of yearling steers in feed lots. An average of 21.7% of the eight groups were infected with *B. sulcata*.

DISCUSSION

Apparently the incidence of *B. sulcata* infection in cattle in the midwestern United States is approximately the same as was found nearly 35 years ago (2). We found 21.7% of 108 cattle from eight different herds in three regions of Nebraska were infected with *B. sulcata*, compared to the 28% incidence reported by Becker and Frye (2) in 1927.

Table 1. Incidence of *B. sulcata* in cattle from three regions in Nebraska.

Location in State	Age of Cattle	Number Examined	Percent Positive
Eastern	Adult cows	12	42
Eastern	3 month calves	12	8
Central	Adult cows	12	8
Central	Yearlings	12	33
Central	Yearlings	12	58
Central	Yearlings	12	25
Central	Feed lot yearlings	12	0
South	Feed lot yearlings	24	0
TOTAL		108	21.7

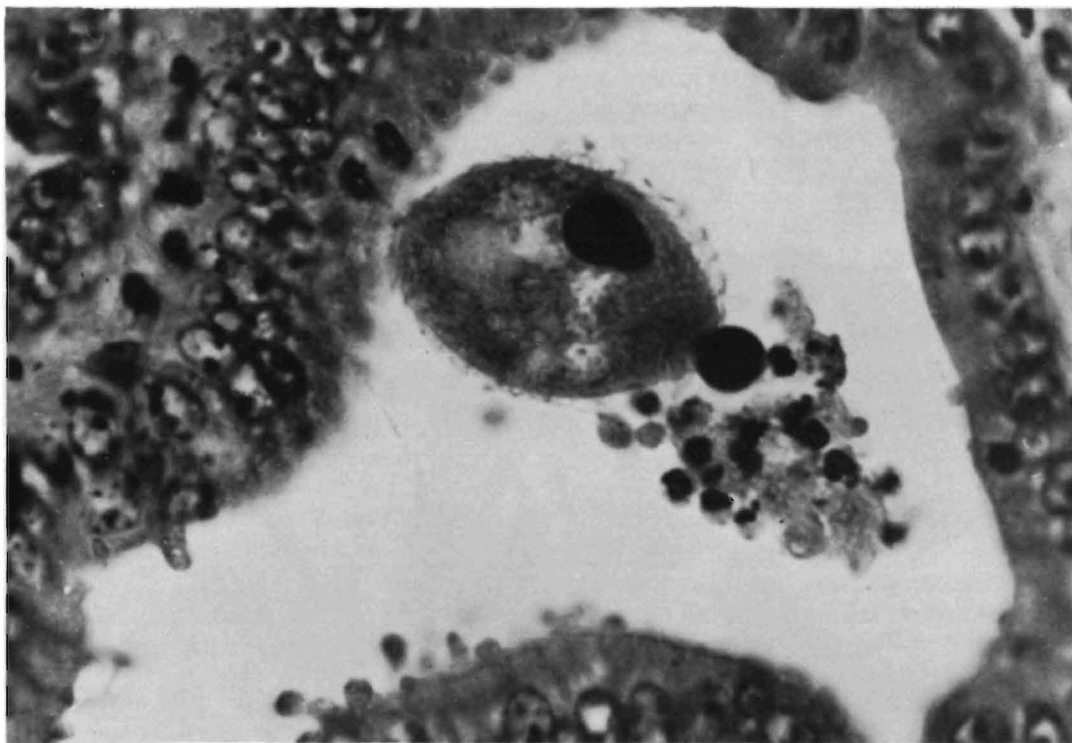


Figure 3. A single parasite showing cilia and other cellular detail. 500X

The presence of *B. sulcata* in feces of cattle has apparently been overlooked in the numerous surveys of cattle parasitism that have been conducted in the U.S. The cyst rapidly changes in appearance upon standing or when recovered by concentration methods using hypertonic solutions; the nuclei can no longer be discerned. For this reason we used three methods to examine fecal specimens, and the specimens were refrigerated until examination.

It is generally assumed that *B. sulcata* is not very pathogenic in cattle (5). *Balantidium coli* produces dysentery in man under some circumstances, but the presence of balantidium alone is not enough to induce pathologic effects in the intestine (1). *B. coli* produces hyaluronidase which is able to dissolve the ground substance between the cells leading to ulceration (7).

B. sulcata was found associated with ulcerative colitis but one cannot draw a definite conclusion as to pathogenicity from this single case. Multiple colonic ulcers with histolymphocytic infiltration occurred in association with the parasites in the mucosa of the large intestine in this case. Balantidial dysentery in man is characterized by lesions infiltrated with numerous eosinophils and round cells but lacking in polymorphonuclear neutrophils (1). Our case showed a paucity of polymorphonuclear neutrophils, and numerous round cells, but very few eosinophils were associated with the lesions. Additional evidence of pathogenicity was the presence of red blood cells and cellular debris within the parasites.

Manlov (4) found that *B. coli* migrate post mortem; *B. sulcata* behaves similarly since we found them singly or in groups lying freely in the serosa. The



Figure 4. Protozoa filled with PAS-positive granules. 500X

migration had occurred post mortem because no inflammatory reaction was present. Parasites found in the serosa contained the same kind of cellular debris as was found within the ciliates in the ulcers.

The protozoa in the sections averaged $53\ \mu \times 31\ \mu$ and in fresh preparations trophozoites had mean measurements of $92\ \mu \times 83\ \mu$. Levine (3) gave measurements for *B. sulcata* of $60\text{--}138\ \mu \times 46\text{--}100\ \mu$. Our observed living trophozoites fit this range, but the sectioned protozoa are too small. Reliable measurements are difficult to obtain in stained sections because the organisms shrink due to the fixing and staining process and the plane of sectioning might not be exactly through the center.

SUMMARY

Buxtonella sulcata, a large ciliate protozoan, was found associated with ulcerative colitis in a cow from a Nebraska dairy herd. An inflammatory reaction consisting of histiocytes and lymphocytes

with very few polymorphonuclear neutrophils or eosinophils occurring around the ulcers. Red blood cells and cellular debris were present in food vacuoles of the protozoa. Granules of carbohydrate (PAS positive) filled the cytoplasm. The protozoa had probably migrated into the tissues after the host had died. Incidence of infection in other members of the herd was not determined but 21.7% of 108 cattle in eight Nebraska herds were found to be infected.

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